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Melatonin and corticosterone profiles under polar day in a seabird with sexually-opposite activity-rhythms

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Abstract

The 24 h geophysical light-dark cycle is the main organizer of daily rhythms, scheduling physiology and behavior. This cycle attenuates greatly during the continuous light of summer at polar latitudes, resulting in species-specific and even individual-specific patterns of behavioral rhythmicity, but the physiological mechanisms underlying this variation are poorly understood. To address this knowledge gap and to better understand the roles of the hormones melatonin and corticosterone in rhythmic behavior during polar day, we exploited the behavior of thick-billed murres (*Uria lomvia*), a charadriiform seabird with sexually opposite ('antiphase') activity-rhythms on a 24 h cycle during the continuous light of polar summer. Melatonin concentration in the plasma of inactive males was unexpectedly high around midday and subsequently fell during a sudden decrease in light intensity as the colony became shaded. Corticosterone concentration in plasma did not vary with time of day or activity in either sex. While the reasons for these unusual patterns remain unclear, we propose that a flexible melatonin response and little diel variation of corticosterone may be adaptive in thick-billed murres, and perhaps other polar birds and mammals, by stabilizing glucocorticoids' role of modulating energy storage and mobilization across the diel cycle and facilitating the appropriate reaction to unexpected stimuli experienced across the diel cycle while attending the colony.

Keywords: activity rhythm, circadian rhythm, corticosterone, melatonin, polar day, *Uria lomvia*

Abbreviations: CI = confidence interval, EIA = enzyme-immunoassay, GLM = general linear model, LM = linear model, RIA = radioimmunoassay

1. **Introduction**

The 24 h geophysical light-dark cycle promotes the appropriate scheduling of behavioral and physiological processes for most organisms (Pittendrigh, 1993; Schwartz and Daan, 2017). When the light-dark cycle is weak, such as during the continuous light of polar summer or continuous darkness of polar winter, a variety of behavioral and physiological patterns have been reported in both free-ranging and captive animals (e.g., free-ranging: Bulla et al., 2016; Steiger et al., 2013; captive: Reierth and Stokkan, 1998; both free-ranging and captive: van Oort et al., 2007). Some organisms under these polar conditions maintain rhythmic behavior (e.g., free-ranging: Ashley et al., 2013; Silverin et al., 2009; Steiger et al., 2013), while others do not (e.g., Reierth and Stokkan, 1998; Steiger et al., 2013; van Oort et al., 2007). The physiological mechanisms of such differences remain unclear (Williams et al., 2015).

Here, we studied melatonin and corticosterone (the primary glucocorticoid in birds), two candidate hormones which have been implicated in 24 h rhythmicity (Dickmeis, 2009; Gwinner et al., 1997; Pevet and Challet, 2011; Son et al., 2011). In most vertebrates they assume stable phase relationships with activity and the light-dark cycle (Gwinner et al., 1997; Landys et al., 2006; Pandi-Perumal et al., 2006; Pevet and Challet, 2011). Melatonin concentration is generally high during the dark phase and is suppressed by light (Gwinner et al., 1997; Pandi-Perumal et al., 2006), and, in birds, changes in melatonin can convey information about diel change in light intensity (Kumar et al., 2000). Glucocorticoids, on the other hand, commonly link with activity and feeding and modulate energy storage and mobilization (Jessop et al., 2002; Landys et al., 2006; Quillfeldt et al., 2007; Woodley et al., 2003). The diel rhythm of baseline corticosterone concentration in birds typically increases during the inactive phase and decreases during the active phase (Breuner et al., 1999; Landys et al., 2006; Romero and Remage-Healey, 2000;

67 Schwabl et al., 2016; Tarlow et al., 2003). Given the above, melatonin can be a physiological
68 marker of the light-dark cycle while corticosterone may be a marker of activity and feeding
69 cycles.
70 To investigate the association of melatonin and corticosterone with the persistence of
71 behavioral activity-rhythms in an environment with a highly attenuated light-dark cycle, we
72 studied the thick-billed murre (a.k.a. Brünnich's guillemot, *Uria lomvia*), a charadriiform seabird
73 that has a sexually segregated ('antiphase') activity rhythm with a duration of 24 h during the
74 continuous light of polar summer (Huffeldt and Merkel, 2016). Thick-billed murres breeding on
75 cliff faces at high latitude are conspicuously rhythmic in their behavior: the inactive mate attends
76 the nest while the active mate forages and provisions their chick (Elliott et al., 2010; Huffeldt
77 and Merkel, 2016). Importantly, the sex that is active diurnally or nocturnally can differ between
78 colonies (Elliott et al., 2010; Huffeldt and Merkel, 2016; Linnebjerg et al., 2015; Paredes et al.,
79 2006; Young et al., 2015), indicating that these birds have a highly plastic circadian system that
80 enables 24 h timekeeping during polar day.
81 During thick-billed murres' 'inactive' phase of their foraging and nest attendance rhythm,
82 they primarily incubate their egg or brood their chick and generally spend little time away from
83 the colony or off the nest around their breeding site (see supplementary actograms in Huffeldt
84 and Merkel, 2016). Additionally, thick-billed murres primarily rest when incubating and
85 brooding (pers. obs.), similar to their congener, the common murre (*U. aalge*; Kappes et al.,
86 2011). These bouts of locomotor-inactivity could be used for essential physiological processes
87 associated with rest. We, therefore, refer to this incubating and brooding state as 'inactive'.
88 Murres breeding above the polar circle under continuous light, however, may need to respond to
89 disturbances from predators and conspecifics around the clock (e.g., Daan and Tinbergen, 1979),

and murres will spend time on the sea surface, potentially resting (Linnebjerg et al., 2014). Hence, we do not know whether the observed antiphase activity rhythm of incubating and brooding associates with hormonal rhythms that also generally follow a 24 h locomotor-activity cycle. We tested the assumption that the activity phases described here associate with corticosterone in the studied population of thick-billed murres (see below).

Our system allowed us to decouple the light-dark cycle from the activity cycle of thick-billed murres. This was possible because each sex was active at opposite times of day when ambient light intensity was also opposite. We, therefore, tested the hypothesis that the sexes had opposite concentrations of circulating melatonin because of the contrasting light environment to which they were exposed at the colony. We predicted that inactive males would have a lower melatonin concentration than inactive females, because males were incubating and brooding at the colony when light intensity was high in the general environment and females were incubating and brooding when light intensity was low. Additionally, we tested the assumption that the diel change in light intensity during polar day was sufficient to affect circulating melatonin. This was possible because the breeding sites studied here were on an east-northeast-facing, vertical cliff and fell suddenly into shade around midday, starkly different from the inverse 'U' shaped profile in the general milieu (Fig. 1a,b). We predicted that the light of the polar day suppressed melatonin secretion until midday, and after that time the dramatically lower level (yet still > 1,000 lx; Fig. 1a,b) of illumination released suppression in inactive birds attending the colony.

Diel rhythms of baseline corticosterone in birds correlate with their activity rhythm and have a pre-activity peak in which circulating corticosterone elevates just before the onset of activity (Breuner et al., 1999; Landys et al., 2006). Therefore, we hypothesized that the activity rhythm of thick-billed murres represented a locomotor-activity and feeding rhythm, and we predicted an

elevation in corticosterone concentration in inactive thick-billed murres in the hours preceding the active phase. To test the assumption that corticosterone associated with activity in thick-billed murres, we sampled circulating corticosterone in provisioning and brooding murres at opposite times of day.

2. Material and Methods

2.1 Study site and fieldwork

We studied breeding thick-billed murres on Kippaku, Greenland (73.72 ˚N, 56.62 ˚W) from the 19th to 28th of July, 2014 and the 22nd to 26th of July, 2017. Light intensity was measured from the 24th to 31st of July, 2016. Birds were captured from a selection of five sampling sites that were within 1 to 10 m vertically from the top of the cliff edge, were visually separated, and spanned approximately 100 m horizontally on the east-northeast-facing side of the breeding cliff. All birds were captured from the side of the breeding cliff using extendable noose-poles, and handling of the birds occurred out of sight of other birds at the sampling site. Blood samples were obtained from the brachial vein following Romero and Reed (2005); all baseline concentrations of corticosterone were obtained from samples collected within 3 min of capture (mean ± sd = 2.0 ± 0.43 min; Supplemental Corticosterone Analysis). When plasma volume was too low to complete both hormone assays (< 80 µL), samples for melatonin were prioritized. Sex was unknown to us during sampling and was identified molecularly from blood or feathers (Griffiths et al., 1998). This study was completed in accordance with Greenlandic law - with approval by the Agency of Fisheries, Hunting, and Agriculture (Dok. nrs. 1565772, 1601149) and Wake Forest University's Animal Care and Use Committee (Protocol: A14-088).

In 2014, blood samples were collected from 49 stationary individuals that were inactive (i.e., incubating their egg or brooding their chick). One blood sample was collected from each individual; blood samples were collected on multiple days over the full 24 h cycle; and a minimum of 10 h elapsed between sampling events from the same sampling site. A sampling event in 2014 consisted of drawing blood from two birds captured from the same sampling site, and a minimum of 20 min separated the release of one bird and the capture of the second in a sampling event. Capture order did not affect corticosterone concentration (Supplemental Table S1). Whole blood was kept below 5 ˚C and centrifuged ≤ 4 h after being drawn. Plasma was separated and then frozen immediately in a liquid nitrogen dry-shipper.

In 2017, blood samples were collected from 27 chick-rearing individuals that were either provisioning (indicated by arrival at the breeding site with a prey item held in the beak; 'active') or brooding their chick ('inactive') to address the effects of activity phase on corticosterone concentration. In most cases, a provisioning bird was sampled and then a brooding bird was sampled in the same sampling event. Captures of birds during the same sampling event occurred at different sites, which were out of view of one another. Four individuals were sampled in both 2014 and 2017; previous capture did not affect the corticosterone concentration in the birds studied (Supplemental Table S1). Sampling occurred within ± 2 h of 12:00 or 24:00, respectively. These times represent the approximate peak and trough of each sex's colony-attendance cycle (Huffeldt and Merkel, 2016). After treating blood as described above, the plasma was removed and then preserved immediately in 100% ethanol (Goymann et al., 2007). To validate the efficacy of the two different methods used to preserve plasma in this study, we sampled six brooding birds on the 26th of July, 2017, in which the plasma from each bird was separated and then a portion (≥ 60 μL) of the sample was preserved in ethanol and another

158 portion (≥ 60 µL) was frozen. All these captures occurred within 59 min of each other, and the
159 captures alternated among three different sampling sites. Samples from these six individuals
160 were not used in additional analyses involving corticosterone, because we did not ensure that we
161 obtained baseline corticosterone concentration from these individuals (e.g., blood was drawn in >
162 3 min after capture, individuals were captured immediately after sampling of another bird within
163 sight of the bird sampled).
164 In 2016, HOBO Pendant light loggers (Onset Computer Corporation, USA) were deployed to
165 measure changes in light intensity every 10 min over the diel cycle on the cairn atop Kippaku
166 and within the colony approximately 6 m below the cliff edge near the sampling sites. The sun
167 never fell below the horizon during fieldwork (range of sun angle at solar midnight = 2.2 to 4.8˚,
168 solar noon = 34.3 to 37.1˚ [USNO]). Time of day is reported in local time: West Greenland
169 Summer Time (WGST, UTC -2).
170
171 *2.2 Laboratory analyses*
172 2.2.1 Melatonin
173 The plasma concentration of melatonin was quantified by radioimmunoassay ('RIA') and run
174 in two assays at the Max Planck Institute for Ornithology following the procedures described by
175 Goymann et al. (2008; Supplemental Methods 1). The standard curves and sample concentrations
176 were calculated with Immunofit 3.0 (Beckman Inc., Fullerton, CA, USA), using a four parameter
177 logistic curve fit. The detection limit of each assay was 5.6 pg/mL and 5.5 pg/mL for samples
178 collected in 2014 and 2017, respectively. The intra-assay coefficients of variation of extracted
179 chicken pools were 3.4% and 6.0% for samples collected in 2014 and 2017, respectively. The
180 inter-assay coefficient of variation was 12.0%.

Samples collected in 2017 and stored in ethanol, following the sampling protocol above, could not be satisfactorily validated against the frozen samples for melatonin (preservation method: frozen [median] = 32.72 pg/mL, range = 23.48 to 47.80 pg/mL, ethanol [median] = 219.19 pg/mL, range = 153.63 to 239.82 pg/mL, [Wilcoxon signed-rank test] $V_{6, 6} = 21$, $p = 0.03$; Huffeldt, 2018). As a result, we deemed that ethanol samples could not be compared with frozen samples in this study. We report only melatonin data originating from frozen samples taken in 2014, because preservation by freezing was the more common method reported in the literature and because the values were more similar to the measurements obtained in 2014 and other charadriiforms, seabirds, and polar breeding birds (see discussion section 4.1; e.g., Cockrem, 1991a, 1991b; Helm et al., 2012; Miché et al., 1991; Silverin et al., 2009; Steiger et al., 2013; Tarlow et al., 2003; Wikelski et al., 2006).

2.2.2 Corticosterone

Corticosterone was measured using an enzyme-immunoassay ('EIA') at the Swiss Ornithological Institute following Jenni-Eiermann et al. (2015; Supplemental Methods 1). The intra-assay and inter-assay variation were 15.5% and 9.8%, respectively, for samples collected in 2014, and 2.5% and 6.9%, respectively, for samples collected in 2017.

The measurements to validate the two preservation methods for corticosterone were within the expected variation of the assay and the values were not significantly different (preservation method: frozen = 5.75 ± 5.61 ng/mL, ethanol = 5.61 ± 4.28 ng/mL, [paired t-test] $t_4 = 0.5$, $p = 0.64$, $n = 5$ birds). The detection limit of the assay was 1.21 ng/mL.

2.3 Statistical analyses

Program R version 3.5.1 was used for all statistical analyses (R Core Team, 2018). Values were log-transformed before statistical analyses to meet assumptions of statistical tests, to improve model fit, or both (Supplemental Methods 2). Descriptive statistics, such as means and medians, are of raw, non-transformed data unless noted otherwise. Standard deviations follow reported means unless noted otherwise (mean ± sd).

2.3.1 Analyzing the association of hormone concentrations in inactive murres and time of day

We used two-sample two-tailed t-tests or non-parametric Mann-Whitney U-tests to test for general differences of melatonin and corticosterone concentrations (continuous, depend variables) among the sexes. Sex was an independent, categorical variable.

We used a linear model ('LM') or generalized linear models with a Gamma error structure and an inverse link function ('GLMs') to model the influence of time of day on the hormone concentrations. Including an interaction between time of day and sex in our statistical tests was not possible owing to sample-size constraints. Either melatonin or corticosterone concentration was our response variable. To increase power for statistical analyses, data from 2014 were consolidated into six 4 h bins beginning at 00:00 local time: 00:00 to 3:59, 4:00 to 7:59, 8:00 to 11:59, 12:00 to 15:59, 16:00 to 19:59, and 20:00 to 23:59, respectively. The 4 h bins are denoted in tables and figures by the times of day: 03:00, 07:00, 11:00, 15:00, 19:00, and 23:00, respectively. We used 4 h bins to maintain an adequate temporal resolution to capture the variability caused by the murre's activity rhythm across the diel cycle. Bins with a single concentration for the hormone of interest provided no indication of variation within that bin and were not included in statistical tests regarding time of day. The bins representing time of day were categorical predictor variables in LMs and GLMs. We used an F test to identify the general

influence of a predictor on the response variable for all LMs and GLMs. We used a Tukey's HSD test for multiple comparisons for post-hoc analyses of the LM and the GLMs used to evaluate the influence of time of day on hormone concentrations. The resulting p-values from the Tukey's HSD test were adjusted using Bonferroni's correction to reduce Type I errors (R function: multcomp::glht; Hothorn et al., 2008).

Means and 95% confidence intervals ('CIs') for light intensity measured in 2016 were calculated using the bootstrap percentile method based on 1,000 replications (R functions: boot::boot and boot::boot.ci; Canty and Ripley, 2017). Light intensity was not included in our statistical tests, but the six 4 h bins, representing time of day, allowed for visually comparing light intensity to the hormone concentrations. We used this indirect comparison because the range of dates used for blood sampling in 2014 was longer and earlier than the date range of light intensity measurements from 2016. Additionally, we used this indirect comparison because the horizontal distribution of the sampling sites along the cliff face probably resulted in variation of the light intensity perceived by individual birds that was not captured by the single location used for measuring light.

2.3.2 Analyzing the association of corticosterone and activity

For the 2017 data, corticosterone concentration was the dependent variable, and the categorical variable 'activity type' (1 = active, 2 = inactive) was the independent variable. We used a two-sample two-tailed t-test to identify if mean corticosterone concentration was different between the activity states.

3. Results

3.1 Light intensity

The east-northeast-facing portion of the cliff face where sampling occurred became shaded as the angle of the sun shifted during the diel cycle, causing an abrupt decrease in light intensity within the 11:00 bin (Fig. 1a,b). In contrast, the change in light intensity atop Kippaku had an inverse 'U' shaped profile (Fig. 1a,b). The range of light intensities measured within the colony was 689 to 209,424 lx and atop Kippaku was 872 to 143,290 lx.

3.2 The association of hormone concentrations in inactive murres and time of day

3.2.1 Melatonin

In 2014, mean melatonin concentration was 40.56 ± 23.71 pg/mL (N = 18 males, 23 females; Supplemental Table S2a). Sex did not generally influence melatonin concentration in inactive birds (sex: male = 42.28 ± 28.38 pg/mL, median = 27.89 pg/mL, range = 16.75 to 92.64 pg/mL; female = 39.21 ± 19.9 pg/mL, median = 31.93 pg/mL, range = 18.24 to 97.89 pg/mL; [Mann-Whitney U-test] $U_{18, 23} = 236$, $p = 0.46$). Melatonin concentration in males was influenced by time of day (time of day: [GLM] $F_{2, 13} = 4.22$, $p = 0.04$; Fig. 1c; Supplemental Table S3a). A Tukey's HSD test for multiple comparisons indicated that melatonin concentration fell significantly between the 11:00 bin and the 15:00 bin in males (Table 1a; Fig. 1c). Time of day did not influence melatonin concentration of females (time of day: [GLM] $F_{3, 18} = 0.26$, $p = 0.85$; Table 1b; Fig. 1e; Supplemental Table S3b).

3.2.2 Corticosterone

In 2014, baseline corticosterone concentration was measured in 41 inactive individuals (N = 20 males, 21 females). Mean corticosterone concentration was 4.97 ± 2.91 ng/mL (Supplemental

Table S2b). Neither time of day nor sex influenced corticosterone concentration significantly (sex: male = 4.91 ± 3.27 ng/mL, female = 5.03 ± 2.59 ng/mL, [t-test] $t_{36.69} = 0.53$, $p = 0.6$; male and time of day: [GLM] $F_{2, 15} = 0.79$, $p = 0.47$; female and time of day: [LM] $F_{3, 16} = 1.47$, $p = 0.26$; Table 2; Fig. 1d,f; Supplemental Table S4).

We found no effect of breeding stage, previous capture (as indicated by a previously deployed ID ring), or capture order for the inactive birds on corticosterone concentration (Supplemental Table S1a). We also found no effect on corticosterone concentration of the amounts of time between initial disturbance and physical capture, between physical capture and the end of blood sampling, or between initial disturbance and the end of blood sampling (i.e., total disturbance; Supplemental Corticosterone Analysis). Furthermore, corticosterone concentration was not affected by an interaction between these variables and time of day (Supplemental Corticosterone Analysis).

3.3 The association of corticosterone and activity

In 2017, the mean corticosterone concentration was 2.85 ± 1.22 ng/mL (N = 18 individuals, Supplemental Table S2b), and this was lower than the mean corticosterone concentration measured in 2014 (4.97 ± 2.91 ng/mL; Supplemental Table S2b). Our direct comparison of provisioning (N = 10) and brooding (N = 8) birds sampled in 2017 indicated that behavioral state did not affect mean corticosterone concentration (provisioning = 2.53 ± 0.9 ng/mL, brooding = 3.26 ± 1.49 ng/mL, $t_{15.31} = -1.3$, $p = 0.21$; Fig. 2).

4. **Discussion**

None of our predictions for the associations of melatonin and corticosterone with time of day, light intensity, and behavioral activity in thick-billed murres was fully supported. A time of day effect was, however, observed for melatonin in incubating and brooding males: melatonin fell in males after midday after light intensity dropped with the onset and continuation of shade on the cliff face (Fig. 1a,b,c,e; Table 1a). A change in melatonin concentration in inactive females was not found. Females were incubating and brooding at a different time of day (at "night"), when there was no sudden drop in light intensity, and their melatonin concentration varied little during their inactive phase (Fig. 1e). The observed decrease in melatonin concentration in males with decreasing light intensity supported our assumption that the change in light intensity during polar day was sufficient to affect melatonin concentration in thick-billed murres. However, the observed effect was opposite to our expectation; we discuss this further below (section 4.1). Corticosterone was associated with neither activity nor time of day (Fig. 1d,f; Fig. 2; Table 2).

We cannot rule out the possibility that time of day had an effect on both melatonin and corticosterone, which we were unable to detect because our sample sizes were small and the variability was high. Additionally, obtaining measurements for light intensity in 2016 at a single location and during a different time-period than that from which the melatonin and corticosterone concentrations were obtained in 2014 excluded a direct comparison among diel changes in light intensity and hormone concentrations (see methods, section 2.3.1). We do not expect that this incongruity affected our interpretation of our results because the overlapping dates of the hormone and light measurements, combined with the summarizing of the diel change in light intensity by means and CIs, captured the general pattern and timing of changes in light intensity within the colony during our study. Additionally, the opportunistic sampling used to address the differences between active and inactive birds did not capture the full temporal

variability of a corticosterone rhythm. The results, however, indicate no fundamental difference in corticosterone concentration between active and inactive murres (Fig. 2) and illustrate that circulating melatonin in inactive males dropped during midday, around the time the breeding cliff became shaded (Fig. 1a,b,c).

4.1 Melatonin

The low mean concentration of melatonin (40.56 ± 23.71 pg/mL) was similar to that known for other charadriiforms (shorebirds [Helm et al., 2012; Steiger et al., 2013] and gulls [Wikelski et al., 2006]) and for non-charadriiform seabirds (Nazca boobies, *Sula granti* [Tarlow et al., 2003] and penguins [Cockrem, 1991a, 1991b; Miché et al., 1991]). However, the melatonin profiles that we detected, particularly in males, were opposite to our expectation that melatonin would be suppressed when the light level was high and would increase when the light level dropped (Ashley et al., 2013; Silverin et al., 2009; Steiger et al., 2013). These results suggested that diel changes between light and dark alone did not control thick-billed murres' diel melatonin rhythms. We speculate that a sudden change in melatonin concentration in response to shade and to the subsequent continuing light may indicate a sensitive and flexible melatonin response in thick-billed murres (cf. Buxton et al., 2000; Underwood and Calaban, 1987). The drop in the melatonin concentration of incubating and brooding males could counter the suppressive effects of melatonin on behavior because of a need to respond to daylight-typical stimuli during this period, such as depredation attempts and conspecific interaction.

Melatonin concentration increased in variability in males during the 19:00 bin and females during 23:00 bin (Fig. 1c,e). In males the increased variability was towards the end of the inactive phase, while in females this increased variability was at the beginning. These periods of

increased variability could have indicated periods of rapid change in melatonin concentration in response to changes in the behavioral state of the birds. This was supported by evidence that melatonin changes with behavioral state in diurnal vertebrates (Jessop et al., 2002; Kumar et al., 2000) and corresponds to decreases in activity in other polar birds (Ashley et al., 2013; Silverin et al., 2009). Additionally, similar physiological responses by each sex to high light-intensity, or to a sudden change in light intensity, could explain why variation did not increase during the earlier behavioral transition of the sexes during the 7:00 and 11:00 bins (Fig. 1a,b,c,e).

The surprising results from our study require further investigation. The missing data caused by the birds foraging away from the colony inhibited the full elucidation of each sex's melatonin profile. Males and females could have had an elevated melatonin concentration during their active phase, which would have suggested a cyclic melatonin profile with a high concentration during activity; this would have, however, contradicted the often negative association between activity and melatonin concentration in diurnal species (Ashley et al., 2013; Jessop et al., 2002; Kumar et al., 2000; Silverin et al., 2009). Thick-billed murres can also spend a significant amount of time on the sea surface (Linnebjerg et al., 2014, 2015), which may include periods of rest, and how this possibly interacted with a flexible melatonin response is unknown.

4.2 Corticosterone

The mean concentrations of baseline corticosterone for thick-billed murres measured in this study (2014 = 4.97 ± 2.91 ng/mL, 2017 = 2.85 ± 1.22 ng/mL) were similar to previously described values for the species (Barger and Kitaysky, 2012; Benowitz-Fredericks et al., 2008), and they fall near those described for common murres (Kristensen et al., 2013) and within the range of 41 species of tropical passerines (Schwabl et al., 2016). Contrary to general

364 expectations, corticosterone varied little and was not associated with activity type (Fig. 1d,f; Fig.
365 2; Table 2; e.g., Breuner et al., 1999; Jessop et al., 2002; Landys et al., 2006; Quillfeldt et al.,
366 2007; Steenweg et al., 2015; Woodley et al., 2003). However, the corticosterone results matched
367 those from some, but not all, species studied under continuous polar light. Adélie penguins
368 (*Pygoscelis adeliae*) and common eiders (*Somateria mollissima*) during summer near the polar
369 circle had no diel variation in circulating corticosterone (Steenweg et al., 2015; Vleck and van
370 Hook, 2002). In the eiders, the lack of diel variation in corticosterone was attributed to
371 continuous activity across the diel cycle in the population studied (Steenweg et al., 2015).
372 Weddell seals (*Leptonychotes weddellii*) gave a similar result for cortisol during polar day
373 (Barrell and Montgomery, 1989). In contrast, a recent study of droppings of barnacle goslings
374 (*Branta leucopsis*) detected weak diel rhythmicity in corticosterone metabolites (Scheiber et al.,
375 2017).
376 We found no indication that our capture protocol influenced the measured baseline
377 concentration of circulating corticosterone (Supplemental Table S1, Supplemental
378 Corticosterone Analysis). We concluded this because no corticosterone stress-response was
379 measureable within the time elapsed between initiating capture and the end of blood sampling
380 and because capture order did not influence corticosterone concentration (Supplemental Table
381 S1, Supplemental Corticosterone Analysis). This was similar to the closely related tufted puffin
382 (*Fratercula cirrhata;* Williams et al., 2008) and differed from the robust corticosterone stress-
383 responses reported for thick-billed murres (Benowitz-Fredericks et al., 2008) and other seabirds
384 and Arctic-breeding birds (Arctic-breeding birds: Wingfield et al., 1995; seabirds: Cape petrels,
385 *Daption capense* [Angelier et al., 2013]; Nazca boobies [Grace and Anderson, 2018]). We

discuss our capture protocol and corticosterone further in the Supplemental Corticosterone Analysis.

Our findings suggested that the corticosterone rhythm was attenuated or absent in thick-billed murres during polar day. It is possible that this attenuation was a result of the continuous light during the polar summer. Near the equator Nazca boobies maintained a diel profile of corticosterone, but the nocturnal rise disappeared under full moon conditions (Tarlow et al., 2003), while penguins and eider ducks residing under continuous light lacked diel variation in corticosterone (Steenweg et al., 2015; Vleck and van Hook, 2002; cf. the barnacle goslings, Scheiber et al., 2017). At least in some species, continuous light might directly or indirectly abolish diel rhythms in corticosterone. As a result, corticosterone's role of modulating energy storage and mobilization may be stable across the diel cycle during polar day.

Little variation across the diel cycle and among the studied activity types could also be explained if the birds were actually active during their presumed inactive phase. This could have prohibited the hormones from reaching concentrations associated with inactive rest. This would suggest that the invariant corticosterone concentration across the diel cycle and among activity types in this study may facilitate reaction to stimuli when attending the colony, which could be complemented by a flexible melatonin response that allows for facilitating rest or sleep during periods of perceived darkness (i.e., through behavioral modulation of perceived light intensity). The use of data loggers, such as an accelerometer coupled with a depth sensor, could elucidate whether the incubating and brooding rhythm represents a true locomotor-activity rhythm of thick-billed murres, and whether the activity rhythm would permit cycling of physiological processes associated with inactive rest.

4.3 Alternative timing cues for polar-breeding thick-billed murres

The absence of clear effects of light on the hormonal rhythms suggested that other environmental timing-cues, such as other solar cues, temperature, or social cues, might be important for synchronizing the 24 h activity-rhythm in thick-billed murres (Ashley et al., 2013; Williams et al., 2015). Other solar cues and temperature are expected to follow a similar diel profile as light, and they could, therefore, be predictable timing-cues in polar environments (Ashley et al., 2013; Williams et al., 2015). Because of the expected similar diel profile to light, we did not expect that temperature or other solar cues could add to explaining our data, and we did not measure these cues within the colony for these reasons. However, temperature could combine with solar timing-cues to provide a predictable indicator of the 24 h day (Ashley et al., 2013; Williams et al., 2015). Social cues can entrain circadian rhythms (Bloch et al., 2013; Fuchikawa et al., 2016). This indicates that social interactions among mates, such as allopreening (Takahashi et al., 2017), during predictable changeovers of incubating and brooding bouts could provide a proximate timing-cue for maintaining rhythms under continuous light. Investigating the influence of other timing cues on the maintenance of 24 h activity-rhythms in polar breeding animals can provide insight into the importance of external timing-cues other than light for the maintenance of biological rhythms.

Another geophysical timing-cue in the marine environment is tides. This rhythmic mass-movement of seawater can serve as an indicator for when to forage (Slater, 1976; Woodley et al., 2003). Common murres can use tides to schedule their colony attendance before the onset of incubation and brooding (Slater, 1976). However, tidal rhythms have different durations than diel and circadian rhythms (12.4 h and 24.8 h vs. 24 h, respectively; Tessmar-Raible et al., 2011), and because thick-billed murres have a pronounced 24 h rhythm of incubating and

brooding (Huffeldt and Merkel, 2016), it is unlikely that tides substantially affect this behavioral rhythm. This does not exclude the possibility that tidal rhythms schedule foraging, if the favored tide occurs during each sex's active phase away from the colony. This could be investigated using foraging behavior measured by time-depth-temperature recorders attached to the birds (e.g., Linnebjerg et al., 2014).

5. **Conclusions**

We conclude that in thick-billed murres diel variation of corticosterone may be unnecessary to maintain the 24 h rhythmic behavior, that corticosterone did not associate with the activity types studied, and that melatonin was variable in its diel profile in incubating and brooding males despite the continuous light of polar day. We propose that a possible invariant corticosterone concentration in thick-billed murres under continuous light could complement a flexible mechanism for modulating circulating melatonin. This proposed association between melatonin and corticosterone may be adaptive for responding to unexpected stimuli while incubating or brooding above the polar circle, such as defending their egg or chick from depredation, in particular by gulls (*Larus* spp.; Daan and Tinbergen, 1979; Gilchrist and Gaston, 1997; Johnson, 1938). Obtaining larger samples sizes and comparing diel patterns of melatonin and corticosterone in thick-billed murres at colonies with contrasting sex-antiphase activity-rhythms would further elucidate the association of melatonin with changes in light intensity at the breeding site and illuminate the generality of our results for corticosterone in thick-billed murres. Additionally, subjecting animals living above the polar circles to experimental periods of darkness during polar day is a promising next step in testing the applicability of our results to other polar breeding birds and mammals.

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Tables

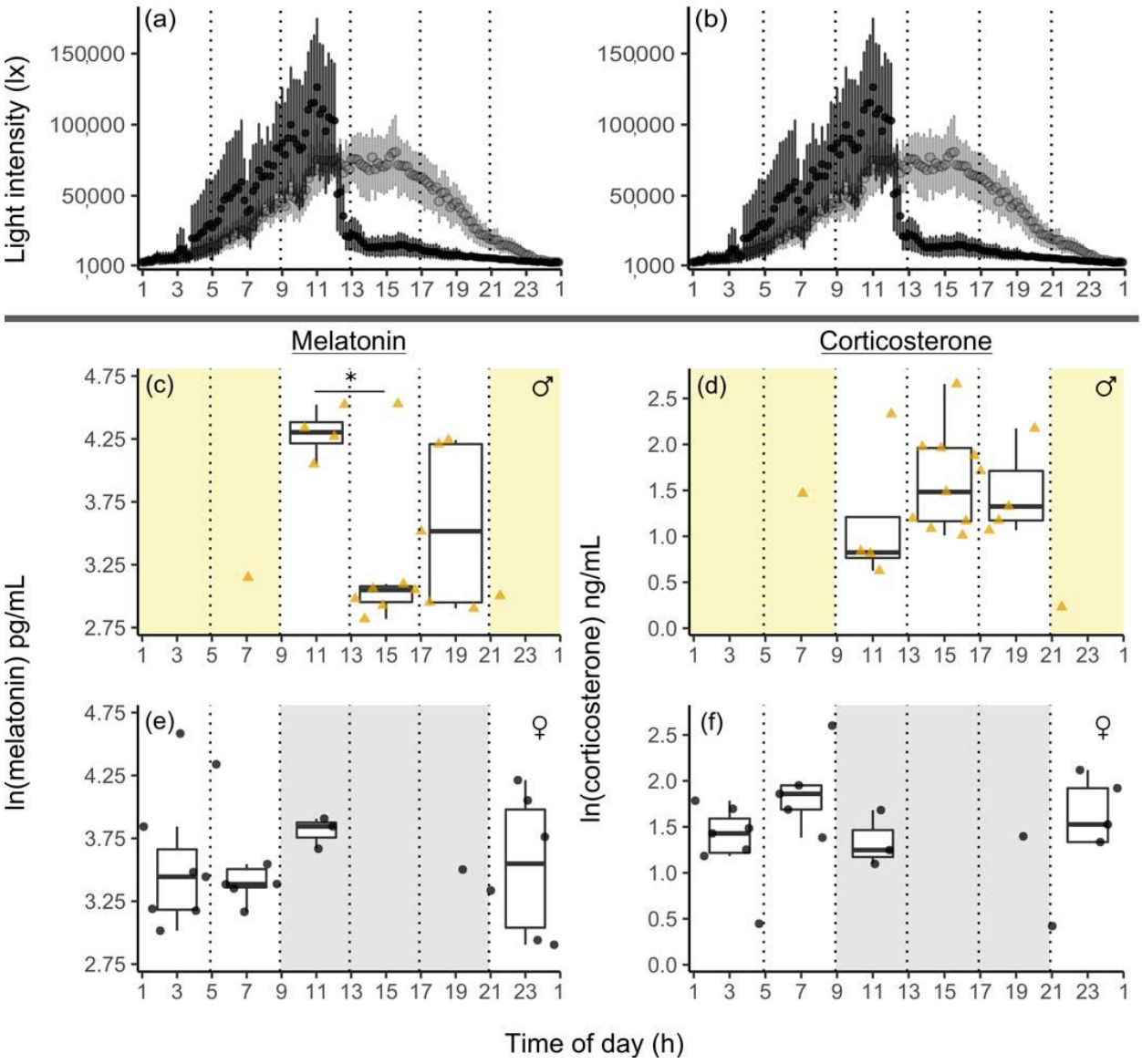
Table 1. Melatonin and time of day. Post hoc comparison of the modeling results of melatonin concentration in males (a) and females (b) among the 4 h bins representing time of day using Tukey's HSD test for multiple comparisons.

	Comparison of 4 h bins	Estimate	Standard error	z-value	Unadjusted p-value	Bonferroni adjusted p-value
(a) males						
	15:00 - 11:00	0.08	0.03	2.93	0.003	0.01
	19:00 - 11:00	0.05	0.03	1.72	0.09	0.26
	19:00 - 15:00	-0.03	0.03	-1.11	0.27	0.80
(b) females						
	3:00 - 11:00	0.02	0.03	0.80	0.42	1.00
	7:00 - 11:00	0.02	0.03	0.79	0.43	1.00
	23:00 - 11:00	0.02	0.03	0.78	0.44	1.00
	7:00 - 3:00	0.0004	0.02	0.02	0.99	1.00
	23:00 - 3:00	-0.00009	0.02	-0.004	1.00	1.00
	23:00 - 7:00	-0.0005	0.02	-0.02	0.98	1.00

Table 2. Corticosterone and time of day. Post hoc comparison of the modeling results of corticosterone concentration in males (a) and females (b) among the 4 h bins representing time of day using Tukey's HSD test for multiple comparisons.

	Comparison of 4 h bins	Estimate	Standard error	z-value	Unadjusted p-value	Bonferroni adjusted p-value
(a) males						
	15:00 - 11:00	-0.24	0.21	-1.18	0.24	0.71
	19:00 - 11:00	-0.20	0.23	-0.87	0.38	1.00
	19:00 - 15:00	0.05	0.16	0.30	0.77	1.00
(b) females						
	3:00 - 11:00	-0.02	0.34	-0.05	0.96	1.00
	7:00 - 11:00	0.55	0.36	1.53	0.14	0.87
	23:00 - 11:00	0.12	0.36	0.34	0.74	1.00
	7:00 - 3:00	0.57	0.29	1.97	0.07	0.40
	23:00 - 3:00	0.14	0.29	0.48	0.64	1.00
	23:00 - 7:00	-0.43	0.31	-1.38	0.19	1.00

692 **Figures captions** (Color figures published online only)



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694 **Figure 1. Diel pattern of light intensity, melatonin, and corticosterone during polar day.**

695 Light intensity, melatonin concentration, and corticosterone concentration from inactive male

696 and female thick-billed murres over the diel cycle during polar day. (a): Mean light intensity with

697 95% confidence intervals measured within the colony near the sampling sites (black) and on the

698 cairn atop Kippaku (grey). (b): plot (a) reprinted for clarity. (c) - (f): Individual data points and

699 box and whisker plots of diel variation of melatonin and corticosterone concentrations in six 4 h

bins for both sexes of inactive thick-billed murres; within each bin, boxes are bound by the first and third quartiles; horizontal bars represent the median; and whiskers represent the smallest or largest measurement within 1.5x the interquartile range. Individual measures of melatonin (c) and corticosterone (d) concentrations from males. Individual measurements of melatonin (e) and corticosterone (f) concentrations from females. Circles (females) and triangles (males) represent individual measurements and the precise time of day in which they were collected. The lighter yellow and darker grey shaded areas represent when males and females, respectively, were primarily active. Vertical dotted lines represent boundaries of the six 4 h bins used for hormone analyses. An * above a horizontal line spanning adjacent bins indicates a statistically significant difference between those bins using a Tukey's HSD test.

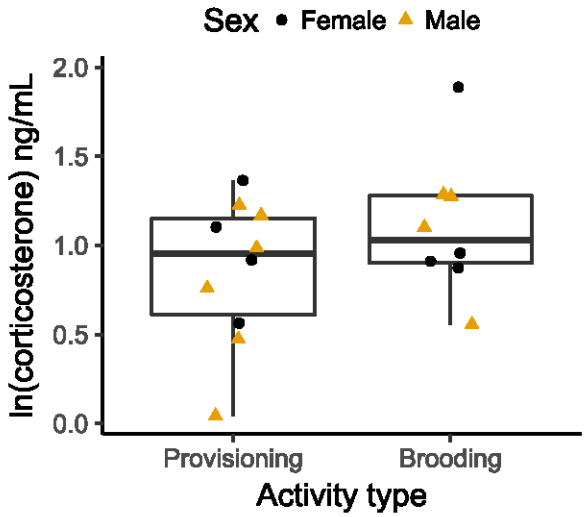

Figure 2. Association of corticosterone with activity type. Individual data points and a box and whisker plot of corticosterone concentration between individuals that are provisioning ('active') or brooding chicks ('inactive'). Details as in Figure 1.

Supplemental Methods 1

Extended description of laboratory analyses

a. Melatonin

Melatonin was extracted with chloroform after overnight equilibration (4 °C) with 1500 dpm of tritiated melatonin (Amersham, Buckinghamshire, UK) to estimate the recovery of extracted melatonin. Then, the extracted samples were dried with nitrogen at 40 °C and re-dissolved in 200 µL of 0.1 M tricine buffer and left overnight at 4 °C to equilibrate. Samples were then washed with petroleum ether to remove residual fats. An aliquot (80 µL) of the re-dissolved samples was transferred to scintillation vials, mixed with 4 mL of scintillation fluid (Packard Ultima Gold), and counted to an accuracy of 2-3% to estimate individual extraction recoveries. Mean (± sd) extraction recovery of melatonin was 77.3 ± 3.4%. The remainder was stored at -40 °C until RIA was conducted. A standard curve was set up in duplicates by serial dilution of stock standard solutions (range = 0.19 to 100 pg). The melatonin antiserum (Stockgrand, LTD: G/S/ 704-8483) was added to the standard curve, the controls, and 100 µL duplicate fractions of each sample. Then, tritiated melatonin label was added and samples incubated for 20 h at 4 °C. Bound and free fractions were separated at 4 °C by adding 0.5 mL of dextran-coated charcoal. After 14 min incubation, samples were spun (3600 g, 10 min, 4 °C), supernatants decanted into scintillation vials at 4 °C, and 4 mL of scintillation liquid was added to each vial.

b. Corticosterone

Corticosterone in 10 µL plasma and 190 µL water (H_2O_{bidest}) was extracted with 4 mL dichloromethane, re-dissolved in phosphate buffer, and measured in triplicates in the EIA. The dilution of the corticosterone antibody (Chemicon; cross reactivity: 11-dehydrocorticosterone 0.35%, progesterone 0.004%, 18-OH-DOC 0.01%, cortisol 0.12%, 18-OH-B 0.02%, and aldosterone 0.06%) was 1:8000. HRP (horseradish peroxidase, 1:400 000) linked to corticosterone served as enzyme label and 2,2 Azino-*bis* (3-ethylbenzo-thiazoline-6-sulfonicacid) diammonium salt (ABTS) as substrate. The concentration of corticosterone in plasma samples was calculated by using the standard curve run in duplicate on each plate. Plasma pool from chicken was included as an internal control on each plate. In 2017, ethanol was evaporated for those samples at 50 °C under a gentle stream of nitrogen. Then the pellet was re-suspended with 2x the volume of water than the original plasma volume and vortexed vigorously. To better dissolve the plasma pellets, the samples were put into an ultrasonic water bath for 15 min. Thereafter, 20 µL instead of 10 µL (due to the dilution), of the re-suspended plasma was extracted and corticosterone was measured following the methods given above.

Supplemental Methods 2

Validation of statistical assumptions and model fit evaluation

i. Identification of normality and homogeneity of variances of log transformed corticosterone concentration

ii. Identification of normality and homogeneity of variances of log transformed melatonin concentration

iii. Diagnostic plots for evaluating the fit of models used for modelling melatonin concentration

iv. Diagnostic plots for evaluating the fit of models used for modelling corticosterone concentration

ii. *Tables 1 and 2.* Identification of normality and homogeneity of variances of log transformed corticosterone concentration

Table 1. Validation of statistical assumptions for analysis of corticosterone data obtained in 2014.

	Shapiro-Wilk normality test		Bartlett test of homogeneity of variances among time-of-day bins		
	W-value	p-value	K^2-value	df	p-value
Both sexes combined	0.99	0.92	3.05	5	0.69
Males	0.94	0.35	1.05	2	0.59
Females	0.96	0.46	1.53	3	0.67

Table 2. Validation of statistical assumptions for analysis of corticosterone data obtained in 2017.

	Shapiro-Wilk normality test		Bartlett test of homogeneity of variances among activity types		
	W-value	p-value	K^2-value	df	p-value
Both activity types combined	0.97	0.77	0.005	1	0.94

iii. *Table 3.* Identification of normality and homogeneity of variances of log transformed melatonin concentration

Table 3. Validation of statistical assumptions for analysis of melatonin data obtained in 2014.

	Shapiro-Wilk normality test		Bartlett test of homogeneity of variances among time-of-day bins		
	W-value	p-value	K^2-value	df	p-value
Both sexes combined	0.92	0.005	3.23	5	0.66
Males	0.82	0.005	3.51	2	0.17
Females	0.96	0.49	3.73	3	0.29

iv. *Diagnostic plots for evaluating the fit of generalized linear models ('GLMs') used for modeling melatonin concentration.*

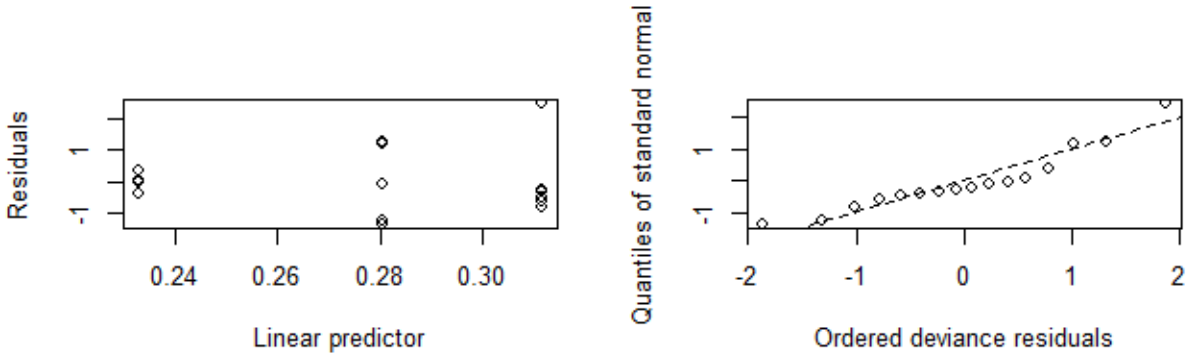

Figure 1. Diagnostic plots for the GLM modeling male melatonin concentration across six 4 h bins. Left: plot of the residuals vs. the fitted values ('linear predictor'). Right: Q-Q plot evaluating if the standardized residuals are normally distributed.

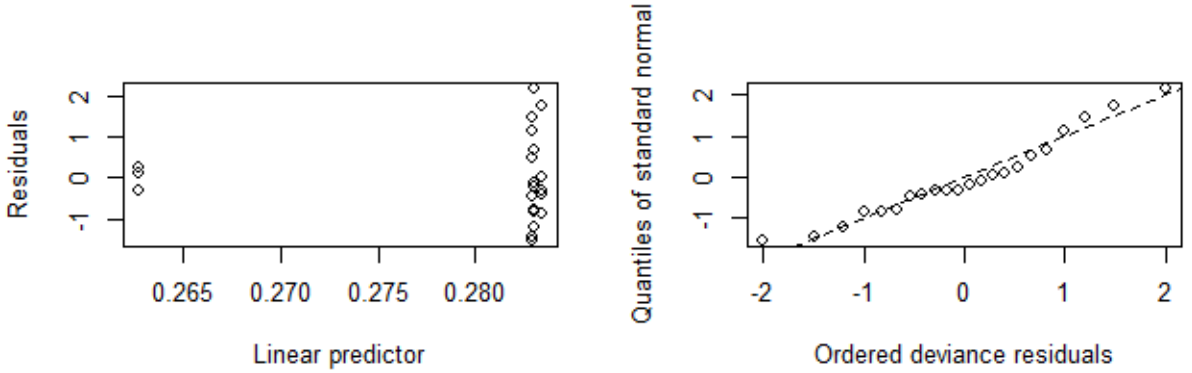

Figure 2. Diagnostic plots for the GLM modeling female melatonin concentration across six 4 h bins. Left: plot of the residuals vs. the fitted values ('linear predictor'). Right: Q-Q plot evaluating if the standardized residuals are normally distributed.

v. *Diagnostic plots for evaluating the fit of the generalized linear model ('GLM') and the linear model ('LM') used for modeling corticosterone concentration*

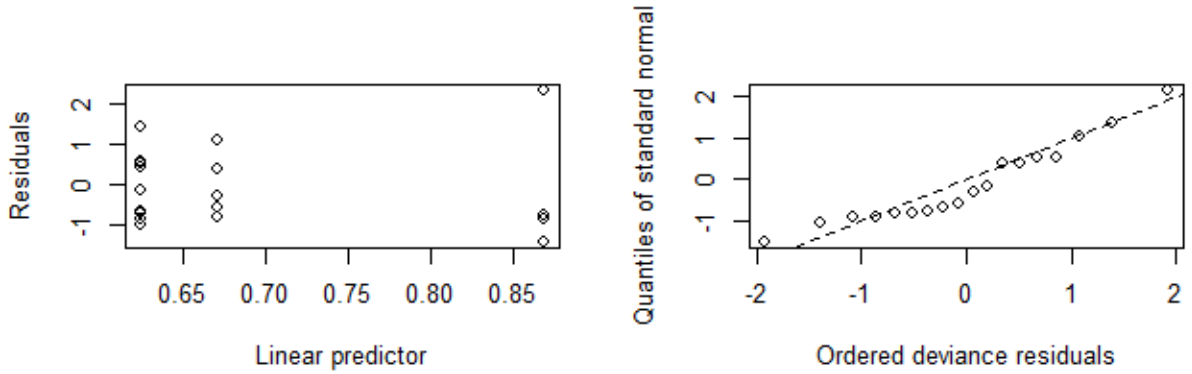

Figure 3. Diagnostic plots for the GLM modeling male corticosterone concentration across six 4 h bins. Left: plot of the residuals vs. the fitted values ('linear predictor'). Right: Q-Q plot evaluating if the standardized residuals are normally distributed.

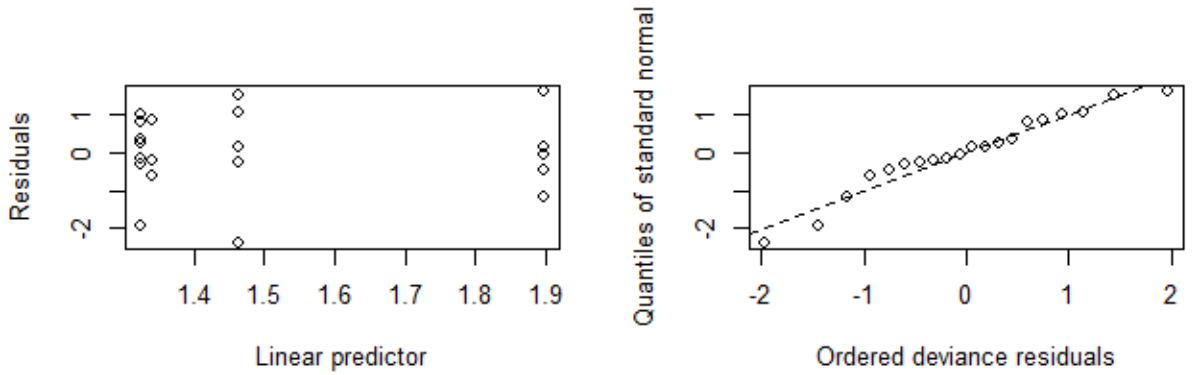

Figure 4. Diagnostic plots for the LM modeling female corticosterone concentration across six 4 h bins. Left: plot of the residuals vs. the fitted values ('linear predictor'). Right: Q-Q plot evaluating if the standardized residuals are normally distributed.

Supplemental Corticosterone Analysis

The influence of the capture protocol on circulating corticosterone concentration.

Because corticosterone is involved in the physiological stress response, identifying if the capture protocol induced a meaningful rise in corticosterone prior to the completion of blood sampling was imperative. Romero and Reed (2005) and Romero and Romero (2002) discuss that sampling blood within 2 min of capture represents baseline concentration of circulating corticosterone in the plasma of birds and that sampling blood within 3 min provides a good representation of baseline concentration. The 3 min cutoff is accepted widely for studying corticosterone in thick-billed murres (*Uria lomvia;* e.g., Barger and Kitaysky, 2012; Benowitz-Fredericks et al., 2008; Elliott et al., 2014), and the closely related tufted puffin (*Fratercula cirrhata*), which is in the same family (alcidae) as thick-billed murres, was stated to not increase its corticosterone concentration before 3 min because of handling (Williams et al., 2008). Here we present data that indicate that our capture protocol did not meaningfully influence the baseline concentration of circulating corticosterone presented in this study.

Our data, following the capture protocol outlined in the main text, addresses three parameters associated with disturbance and capture in thick-billed murres. We tested if a significant rise in baseline corticosterone concentration occurred during our study and if a rise was associated with the duration of disturbance before physical capture (delta disturbance; see 'a' below), the duration of physical capture until the end of blood sampling (delta capture; see 'b' below), or the sum of delta disturbance and delta capture (total disturbance; see 'c' below). This information and these tests exceed what is normally presented in the literature for similar

studies addressing baseline corticosterone (e.g., Angelier et al., 2008; Barger and Kitaysky, 2012; Benowitz-Fredericks et al., 2008; Elliott et al., 2014; Storey et al., 2017). Additionally, we tested if capture order, previous capture, or breeding stage affected baseline corticosterone concentration by using an ANOVA or two-way, two-sample t-test. We used linear models and log transformed data to address the relationship among corticosterone concentration (response variable) and delta disturbance, delta capture, and total disturbance (all continuous predictor variables) and their interaction with time of day (continuous predictor variable). Corticosterone concentration was log transformed to adhere to assumptions of the statistical tests.

No effect was found for delta disturbance (see 'a' below, Fig. S1), delta capture (see 'b' below, Fig. S2), or total disturbance (see 'c' below, Fig. S3) on circulating corticosterone concentration. Time of day did not influence delta disturbance, delta capture, or total disturbance (see below). Baseline corticosterone concentration was not affected by capture order, previous capture, or breeding stage (Table S1 in Supplemental Tables). Although, we cannot rule out that our sampling protocol could mask small changes in corticosterone concentration across the diel cycle, as discussed in the main text, we find it likely that our measurements represent the approximate baseline concentration in thick-billed murres. This is based on the insignificance of the statistical analyses presented here, previous studies that describe 3 min from physical capture as a cutoff for estimating baseline concentration of corticosterone, and because stress induced concentrations of corticosterone in thick-billed murres are approximately 10x the baseline concentration presented in this study (Benowitz-Fredericks et al., 2008; unpubl. data).

a. *Duration of disturbance before physical capture (delta disturbance):*

Delta disturbance was the duration from the first visual contact between the bird being captured and the captor until the noose was placed around the neck of the subject, i.e., physical capture. Circulating corticosterone was neither influenced by the interaction between delta disturbance and time of day (linear model: log(corticosterone) ~ delta disturbance*time of day, adjusted $r^2 = -0.03$, $F_{11, 29} = 0.9$, $p = 0.55$) nor by only delta disturbance (Fig. S1).

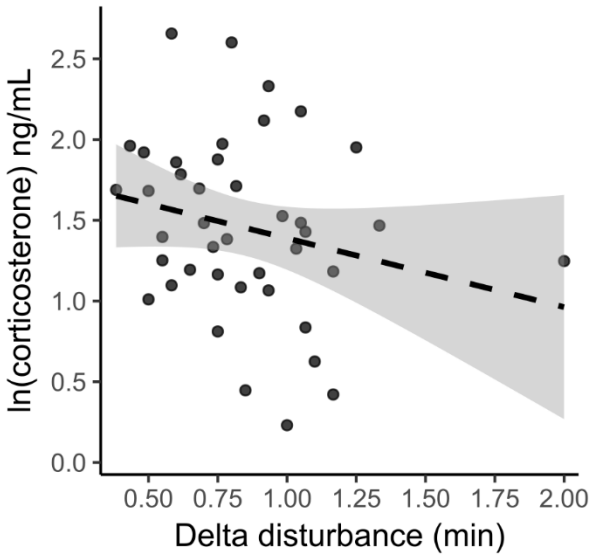

Figure S1. The influence of duration of disturbance before physical capture on corticosterone concentration (linear model: log(corticosterone) ~ delta disturbance, adjusted $r^2 = 0.03$, $F_{1, 39} = 2.2$, $p = 0.15$).

b. *Duration of physical capture until end of blood sampling (delta capture):*

Delta capture was the duration from the placement of the noose around the bird's neck until the end of blood sampling. Circulating corticosterone was neither influenced by the interaction between delta capture and time of day on corticosterone concentration (linear model: log(corticosterone) ~ delta capture*time of day, adjusted $r^2 = 0.08$, $F_{11, 29} = 1.34$, $p = 0.26$) nor by only delta capture (Fig. S2).

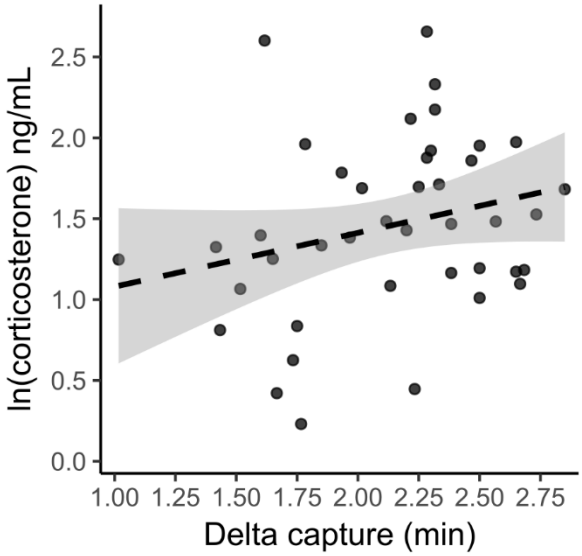

Figure S2. The influence of the duration of physical capture on corticosterone concentration (linear model: log(corticosterone) ~ delta capture, adjusted $r^2 = 0.04$, $F_{1, 39} = 2.79$, $p = 0.1$).

c. *Duration of total disturbance (delta disturbance + delta capture):*

Total disturbance is the sum of delta disturbance and delta capture, i.e., the duration from first visual contact between the subject and the captor until the end of blood sampling. Circulating corticosterone was neither influenced by the interaction between total disturbance and time of day on corticosterone concentration (linear model: log(corticosterone) ~ total disturbance*time of day, adjusted $r^2 < -0.001$, $F_{11, 29} = 1.0$, $p = 0.47$) nor by only total disturbance (Fig. S3).

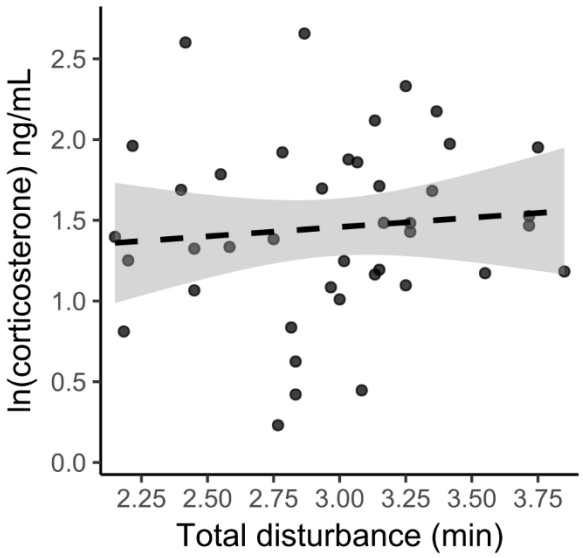

Figure S3. The influence of duration of total disturbance on corticosterone concentration (linear model: log(corticosterone) ~ total disturbance, adjusted $r^2 = -0.02$, $F_{1, 39} = 0.34$, $p = 0.56$).

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915

Supplemental Tables

v. *Supplemental Table S1.* Influence of capture order, breeding status, and if captured previously on corticosterone concentration from egg-incubating and chick-brooding (i.e., inactive) thick-billed murres (*Uria lomvia*)

vi. *Supplemental Table S2.* Median and range of melatonin and corticosterone measurements

vii. *Supplemental Tables S3.* Results from GLMs modeling the influence of the 4 h bins representing time of day on melatonin concentration

viii. *Supplemental Tables S4.* Results from either a LM or a GLM modeling the influence of the 4 h bins representing time of day on corticosterone concentration

vi. *Supplemental Table S1.* Influence of capture order, breeding status, and if captured previously on corticosterone concentration from egg-incubating and chick-brooding (i.e., inactive) thick-billed murres

Table S1. (a) Influence of capture order, breeding status, and if captured previously on corticosterone concentration from egg-incubating and chick-brooding (i.e., inactive) thick-billed murres in 2014 using an ANOVA: aov(log(corticosterone) ~ capture order + previously captured + breeding stage). The presence of an identification ring was used to identify if a bird was captured previously. (b) Results from a two-tailed, two-sample t-test identifying the influence of being previously captured during sampling in 2014 on corticosterone concentration in 2017.

(a) 2014

		mean ± sd log(ng/mL)	n	df	F-value	p-value
Capture order	First	1.38 ± 0.45	22	1	1.01	0.32
	Second	1.55 ± 0.66	19			
Previously captured	No	1.4 ± 0.42	24	1	0.50	0.49
	Yes	1.53 ± 0.71	17			
Breeding stage	Brooding	1.54 ± 0.54	30	1	2.51	0.12
	Incubating	1.21 ± 0.55	11			

(b) 2017

		mean ± sd log(ng/mL)	n	df	t-value	p-value
Previously captured	No	0.93 ± 0.45	14	11.2	-1.03	0.33
	Yes	1.1 ± 0.21	4			

940

941 ***vii.*** *Supplemental Table S2.* Median and range of melatonin and corticosterone measurements

942

943 **Table S2.** Median and range of melatonin and corticosterone measurements from all samples

944 collected in 2014 and 2017. Comparison of corticosterone concentration in 2014 and 2017 using

945 a two-tailed t-test.

Year	Median	Range	t-value	df	p-value
(a) melatonin					
2014	31.33 pg/mL	16.75 to 97.89 pg/mL			
(b) corticosterone					
2014	4.17 ng/mL	1.26 to 14.25 ng/mL	-3.75	43.5	0.0005
2017	2.64 ng/mL	1.04 to 6.61 ng/mL			

946

viii. *Supplemental Tables S3.* Results from GLMs modeling the influence of the 4 h bins representing time of day on melatonin concentration

Table S3. Results from GLMs modeling the influence of the 4 h bins representing time of day on melatonin concentration in male (a) and female (b) thick-billed murres. Reference concentration was the 11:00 bin.

Predictor	Estimate	95% CI	t-value	p-value	N
(a) males					
Intercept (11:00)	0.23	0.20 to 0.27	12.33	< 0.001	4
15:00	0.08	0.03 to 0.13	2.93	0.01	7
19:00	0.05	-0.007 to 0.10	1.72	0.11	5
(b) females					
Intercept (11:00)	0.26	0.22 to 0.31	12.69	< 0.001	3
3:00	0.02	-0.03 to 0.07	0.80	0.43	7
7:00	0.02	-0.03 to 0.07	0.79	0.44	6
23:00	0.02	-0.03 to 0.07	0.78	0.45	6

(a,b) GLM(log(melatonin) ~ 4 h bin)

ix. *Supplemental Tables S4.* Results from either a LM or a GLM modeling the influence of the 4 h bins representing time of day on corticosterone concentration

Table S4. Results from either a LM or a GLM modeling the influence of the 4 h bins representing time of day on corticosterone concentration in male (a) and female (b) thick-billed murres. Reference concentration was the 11:00 bin.

Predictor	Estimate	95% CI	t-value	p-value	N
(a) males					
Intercept (11:00)	0.87	0.55 to 1.29	4.67	< 0.001	4
15:00	-0.24	-0.69 to 0.13	-1.18	0.26	9
19:00	-0.20	-0.67 to 0.23	-0.87	0.40	5
(b) females					
Intercept (11:00)	1.34	0.74 to 1.95	4.70	< 0.001	3
3:00	-0.02	-0.74 to 0.71	-0.05	0.96	7
7:00	0.55	-0.21 to 1.32	1.53	0.14	5
23:00	0.12	-0.64 to 0.89	0.34	0.74	5

(a) GLM(log(corticosterone) ~ 4 h bin)

(b) LM(log(corticosterone) ~ 4 h bin)